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# How GABA generates depolarization

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In a masterful, insightful and influential paper published in *The Journal of Physiology* in 1989, Ben-Ari, Cherubini, Corradetti and Giarsa understood for the first time the basis of the depolarizing action of GABA ($\gamma$-aminobutyric acid) in immature neurons, suggesting that it was due to an elevated level of intracellular chloride ions and efflux through chloride-permeable channels (Ben-Ari *et al.* 1989). GABA had long been recognized as an inhibitory neurotransmitter that hyperpolarizes mature neurons. However Obata and his colleagues (Obata *et al.* 1978) suggested that there is a developmentally regulated shift in the action of GABA, and Mueller *et al.* (1984) reported more negative reversal potentials for GABA in mature than in immature neurons. The ion channels that mediated these different responses were subjects of interest. The paper by Ben-Ari *et al.* was a great advance for the field of developmental neuroscience, spawning a host of studies of the mechanism of reduction in the intracellular concentration of chloride ions and stimulating an array of investigations of the functional implications of early depolarizing responses to GABA.

The study began with recordings of spontaneous giant depolarizing potentials (GDPs) from neurons in slices from the rat hippocampus during the first postnatal week. They were several hundred milliseconds in duration with amplitudes up to 50 mV, and were generated synchronously in a population of neurons. Strikingly, they had depolarized reversal potentials that were the same as those for exogenously applied GABA, and were blocked by bicuculline or picrotoxin. Their frequency was reduced by NMDA receptor antagonists or channel blockers. Nicely, stimulation of the hilus during the first week of life elicited GDPs with properties indistinguishable from the spontaneous events. Tonic depolarization by endogenous GABA was also apparent. During the second postnatal week, when spontaneous GDPs were largely absent, stimulation of the hilus evoked an excitatory postsynaptic potential followed by fast and slow inhibitory postsynaptic potentials, and now bicuculline induced interictal discharges.

The authors were thinking hard about these results:

'Although the present study indicates that a $Cl^-$ permeability increase was likely to be responsible for the action of GABA, it is not fully clear why such an effect of GABA should be depolarizing and thus capable of eliciting GDPs. In adult hippocampal cells, somatic or dendritic application of GABA produces a hyperpolarization and a depolarization respectively'.

Their thinking was prescient:

'Assuming that only $Cl^-$ is involved, it is possible that in immature cells the depolarizing responses to GABA, like those observed following dendritic application of GABA to adult neurones, were due to a modified $Cl^-$ gradient resulting from the unidirectional operation of the $Cl^-$ membrane pumps'.

Several other important conclusions were reached. Neuronal activity at early stages of development is generated by a network that is driven by GABAergic GDPs. Early glutamatergic synapses, in turn, rely largely on postsynaptic NMDA receptors. Fluorescent optical indicators for $Cl^-$ were later used to directly visualize chloride efflux, which decreased with further development (Kuner & Augustine, 2000; Marandi *et al.* 2002). The mechanisms by which GDPs are generated have been further elucidated. GABAergic depolarization promotes voltage-dependent bursting activity of CA3 pyramidal neurons in neonatal rat hippocampal slices and glutamatergic CA3 neurons play a role in the generation of GDPs (Sipilä *et al.* 2005).

The major transporters regulating levels of intracellular $Cl^-$ have been identified. Uptake of $Cl^-$ in immature neurons is mediated by the NKCC1 transporter, which plays a key role in maintaining high intracellular $Cl^-$. KCC2 is the major transporter for $Cl^-$ extrusion from neurons. $Cl^-$ extrusion is modest in immature neurons and increases with neuronal maturation. Transcripts encoding KCC2 are expressed at low levels in the rat hippocampus at birth but increase rapidly at the end of the first postnatal week (Rivera *et al.* 1999). Antisense inhibition of KCC2 expression shifts the reversal potential of $GABA_A$ responses in a depolarizing direction in mature hippocampal pyramidal neurons, leading to the conclusion that developmental expression of KCC2 underlies development of hyperpolarizing inhibition mediated by GABA.

The mechanism regulating the switch in transporter expression appears to be different under different conditions and in different neurons. GABA depolarizes dissociated immature rat hippocampal neurons and stimulates elevation of intracellular $Ca^{2+}$; blockade of $GABA_A$ receptors prevents the normal developmental negative shift in $E_{GABA}$, the loss of $Ca^{2+}$ responses, and suppresses expression of transcripts encoding KCC2 (Ganguly *et al.* 2001). However, blockade of $GABA_A$ receptors has also been observed to have no effect on the developmental increase in KCC2 expression and the negative shift in $E_{GABA}$ in mouse neonatal hippocampal neurons in slice cultures (Ludwig *et al.* 2003). Interestingly, spontaneous nicotinic cholinergic activity has been found to play a key role in terminating GABAergic excitation and initiating inhibition, by changing chloride transporter levels in the mouse neonatal hippocampus *in vivo* (Liu *et al.* 2006). Activation of $K^+/Cl^-$ co-transport by endogenous tyrosine kinases can also mediate the developmental switch in GABA responses (Kelsch *et al.* 2001; Aguado *et al.* 2003). However, amphibian dorsal root ganglion neurons do not switch their responses to GABA (Alvarez-Leefmans *et al.* 1988). The recent discovery that the chloride reversal potential can be regulated by the level of energy substrates (Holmgren *et al.* 2009) identifies a further level of plasticity.

Depolarization by GABA has been found to play numerous roles in the developing nervous system, including regulation of proliferation, migration and differentiation. For example, GDPs play a role in strengthening developing synapses. Activation of mossy fibres coincident with GDPs increases the efficacy of the mossy fibre–CA3 synapse (Kasyanov *et al.* 2004). This combinatorial code for coincidence detection generates an outcome that would



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not be achieved by independent stimulation of mossy fibres and CA3 neurons. Even in the adult nervous system, depolarization by GABA is important for the integration of newly born neurons into existing circuits. Conversion of ambient GABA-induced depolarization of dentate granule cells into hyperpolarization by retroviral expression of short hairpin RNA against NKCC1 leads to defects in their synapse formation and dendritic development (Ge *et al.* 2006). Remarkably, protection against adverse effects of excitation by GABA is available to the fetus. The surge of oxytocin that signals the onset of parturition stimulates a transient reduction in intracellular $Cl^-$ concentration and a switch from excitatory to inhibitory action of GABA. The effect is neuroprotective, because blockade of oxytocin receptors exacerbates the severity of anoxic episodes (Tyzio *et al.* 2006).

It is clear that the 1989 paper by Ben-Ari and colleagues is a classic, both with respect to the insights that it provided and its impact in launching numerous new lines of investigation.

## References


Aguado F, Carmona MA, Pozas E, Aguiló A, Martínez-Guijarro FJ, Alcantara S *et al.* (2003). BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of $K^+/Cl^-$ co-transport. *Development* **130**, 1267–1280.

Alvarez-Leefmans FJ, Gamiño SM, Giraldez F & Noguerón I (1988). Intracellular chloride regulation in amphibian dorsal root ganglion neurones studied with ion-selective microelectrodes. *J Physiol* **406**, 225–246.

Ben-Ari Y, Cherubini E, Corradetti R & Gaiarsa JL (1989). Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol* **416**, 303–325.

Ganguly K, Schinder AF, Wong ST & Poo M (2001). GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell* **105**, 521–532.

Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL & Song H (2006). GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* **439**, 589–593.

Holmgren CD, Mukhtarov M, Malkov AE, Popova IY, Bregestovski P & Zilberter P (2009). Energy substrate availability as a determinant of neuronal resting potential, GABA signalling and spontaneous network activity in the neonatal cortex *in vitro*. *J Neurochem*; DOI: 10.1111/j.1471-4159.2009.06506.x.

Kasyanov AM, Safiulina VF, Voronin LL & Cherubini E (2004). GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. *Proc Natl Acad Sci U S A* **101**, 3967–3972.

Kelsch W, Hormuzdi S, Straube E, Lewen A, Monyer H & Misgeld U (2001). Insulin-like growth factor 1 and a cytosolic tyrosine kinase activate chloride outward transport during maturation of hippocampal neurons. *J Neurosci* **21**, 8339–8347.

Kuner T & Augustine GJ (2000). A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons. *Neuron* **27**, 447–459.

Liu Z, Neff RA & Berg DK (2006). Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. *Science* **314**, 1610–1613.

Ludwig A, Li H, Saarma M, Kaila K & Rivera C (2003). Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. *Eur J Neurosci* **18**, 3199–3206.

Marandi N, Konnerth A & Garaschuk O (2002). Two-photon chloride imaging in neurons of brain slices. *Pflugers Arch* **445**, 357–365.

Mueller AL, Taube JS & Schwartzkroin PA (1984). Development of hyperpolarizing inhibitory postsynaptic potentials and hyperpolarizing response to gamma-aminobutyric acid in rabbit hippocampus studied *in vitro*. *J Neurosci* **4**, 860–867.

Obata K, Oide M & Tanaka H (1978). Excitatory and inhibitory actions of GABA and glycine on embryonic chick spinal neurons in culture. *Brain Res* **144**, 179–184.

Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K *et al.* (1999). The $K^+/Cl^-$ co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* **397**, 251–255.

Sipilä ST, Huttu K, Soltesz I, Voipio J & Kaila K (2005). Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. *J Neurosci* **25**, 5280–5289.

Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hübner CA, Represa A *et al.* (2006). Maternal oxytocin triggers a transient inhibitory switch in GABA signalling in the fetal brain during delivery. *Science* **314**, 1788–1792.